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Nonsense Mutation in Exon 3 of the Proteolipid Protein Gene (*PLP*) in a Family With an Unusual Form of Pelizaeus-Merzbacher Disease

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We report a G→A transition at nucleotide 431 of the proteolipid protein gene (*PLP*) results in a nonsense codon in a family with an unusual form of Pelizaeus-Merzbacher disease (PMD). The mutation, which creates a second *Alu*I restriction site, results in a nonsense mutation in *PLP*. The clinical picture resembles somewhat that of X-linked spastic paraplegia (SPG). It differs from this and both the classical and connatal forms of PMD in that it is relatively mild in form, onset is delayed beyond age 2 years, nystagmus is absent, tremors are prominent, mental retardation is not severe, some patients show dementia or personality disorders, the disease is progressive rather than static in some, and several females show signs of disease. The nonsense mutation, which is in exon 3B, should block the synthesis of normal PLP but spare DM20, the isoform whose persistence has been associated with mild forms of PLP-associated disease in both humans and mice. Am. J. Med Genet. 69:121–125, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: Pelizaeus-Merzbacher disease; proteolipid protein; nonsense mutation; X-linked spastic paraplegia

INTRODUCTION

Pelizaeus-Merzbacher disease (PMD) is an X-linked dysmyelinating disorder of the central nervous system (CNS). The major protein of CNS myelin is proteolipid protein (PLP). Mutations in the *PLP* gene have been linked to and implicated as the cause of PMD and of X-linked spastic parplegia (SPG), a disease with overlapping manifestations [Gencic et al., 1989; Hudson et al., 1989; Trofatter et al., 1989; Hodes et al., 1993]. The onset of classical PMD is usually within a few months of birth. The clinical presentation includes nystagmus, titubation, and psychomotor delay, but in contrast with the connatal form of the disease, death is delayed beyond the second decade of life. In contrast, the most prominent sign of SPG is spastic diplegia, while nystagmus may be absent. Magnetic resonance imaging (MRI) has become an invaluable diagnostic tool because the images show a severe deficiency in or complete lack of normal myelin in PMD patients. We report here a family with onset of disease delayed past the second year of life, tremors as the initial sign, mild retardation that may progress to dementia, neurological signs in some females, prolonged survival and a nonsense mutation in exon 3B.

SUBJECTS

Study Subjects

The propositus (III-3, Fig. 1) is a 22-year-old man who is a graduate of a special education high school. At age 2.5 years, he had onset of tremors which were more prominent during times of stress. A slowly progressive movement disorder affected both the upper and lower limbs, neck and trunk. This was continuous during waking hours exacerbated with stress or anxiety and absent during sleep. At age 14 years, his neurological status was notable for the absence of nystagmus, presence of dysmetria, dysarthria, occasional jerking of the head, eyebrows and mouth, normal tone in the upper and slightly increased tone in the lower limbs. The tremors caused increasing impairment of his ability to write and his school grades fell from B–C to D–F. Psychological testing showed an IQ of 77. He was placed in a class for the emotionally disturbed. He had been treated with Ritalin® and Valium® for his behavior problems. Visual evoked response was grossly abnormal with bilateral delay in the ocular pathway, whereas brainstem auditory-evoked response and EEG were normal. MRI showed generalized white matter dysmyelination, consistent with Pelizaeus-Merzbacher

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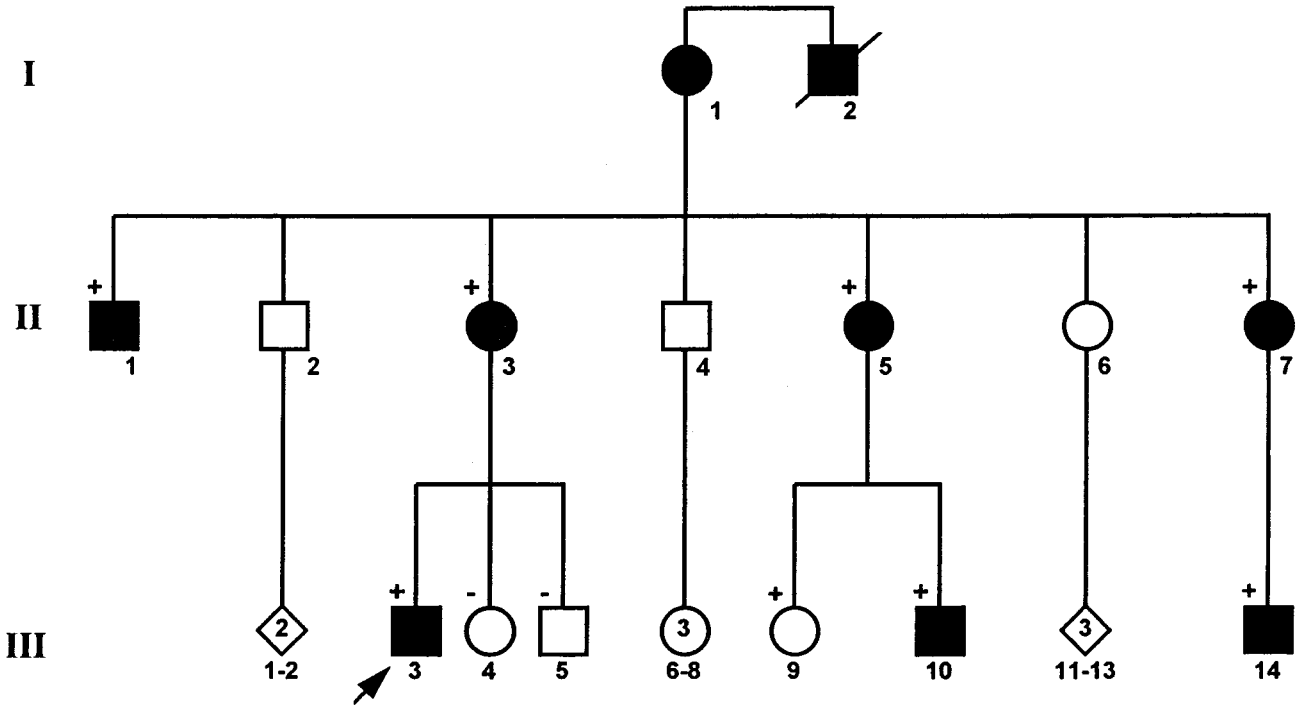

Fig. 1. Abbreviated pedigree of family. Results of DNA examination shown as + (presence of mutation) or − (absence of mutation) above squares (males) or circles (females). Filled in symbols, affected individuals. The arrow points to the propositus.

disease. Metachromatic leukodystrophy and GM1 gangliosidosis were excluded by normal β-galactosidase and arysulfatase A levels.

The family history, gleaned from various sources, is significant in that I-2 was confined to a wheelchair and died in a nursing home at age 36. II-1 had problems similar to those of the propositus and has lived in a nursing home from age 37 years. His current state is "vegetative." Females I-1, II-3, II-5, and II-7 have tremors that began between 8 and 12 years. II-7 is very mildly affected but has an affected male child (III-14) with symptoms much like those of his cousins, III-3 and III-10. III-14 has an attention deficit and has been treated with Ritalin, with questionable results. Head MRI of II-3 showed "leukodystrophy."

A maternal first cousin of the propositus, III-10, was examined when 14 years old. He too had tremors since early childhood, difficulty in writing and in riding a bicycle. There was progression in his voice tremor and speech had become less articulate. He walked well, but gait was wide-based. Motor strength was excellent and neither spasticity nor nystagmus was noted. MRI imaging showed white matter disease and Arnold-Chiari malformation.

The first cousin's mother, II-5, had onset of tremors in approximately the first grade. She attended school through grade 12, the last 4 years in special classes. She has had some ill-defined psychiatric problems. She was able to tandem walk, but gait was wide-based. There were occasional myoclonic-like jerks of the eyebrow, slight head and voice tremor and mild finger-to-nose dysmetria. Her condition has been stable over the past 8 years.

MATERIALS AND METHODS

Molecular Biological Methods

Single strand conformational polymorphism (SSCP) analysis was performed by the protocol of Orita et al. [1989]. The *PLP* exons were amplified and sequenced as described by Pratt et al. [1991]. *Alu*I digestion was done according to manufacturer's recommendations. **S**ingle **n**ucleotide **p**rimer **e**xtension (SNuPE) was done as previously described by Pratt et al. [1993]. The SNuPE primer was 5′-CTTGTCGGGATGTCCTAGC-3′. α-^{32}P dCTP was used to detect the normal allele, α-^{32}P dTTP was used to detect the mutation-containing allele and α-^{32}P dATP was used as a negative control.

Linkage

Linkage was done with the MENDEL program [Lange et al., 1988].

RESULTS

During SSCP screening of the 7 exons of the *PLP* gene, a shift was detected in exon 3 of the propositus's *PLP* gene (Fig. 2). The exon was sequenced and a G→A transition was found at nucleotide 431 (Fig. 3). This transition resulted in a stop codon (TAG) instead of tryptophan (TGG) at amino acid 144. The nonsense mutation created a second *Alu*I restriction site in exon 3 (Fig. 4). The presence or absence of the point mutation in other relatives was tested by SSCP (Fig. 2.)

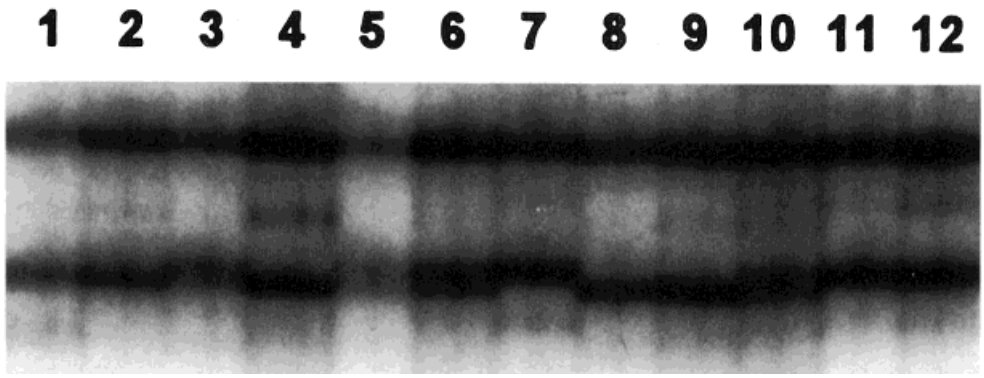

Fig. 2. Single strand conformational polymorphism (SSCP) analysis of amplified genomic DNA from the family. Lanes and individuals designated in pedigree; Figure 1: **1,2**, normal male controls; **3**, II-3; **4**, father of III-3; **5**,II-5; **6**, II-7; **7**, III-3; **8**, III-4; **9**, III-5; **10**, III-9; **11**, III-10; **12**, III-**14**.

and was confirmed by AluI digestion (Fig. 4) and by SNuPE (data not shown).

Passage of the *PLP* nonsense mutation is concordant with PMD in all tested males and most females in this family. Female III-9 (Fig. 1) carries the mutation but does not show signs of disease. We have not encountered this mutation in over 152 X chromosomes examined in individuals unrelated to this family.

DISCUSSION

We have presented here a family with a nonsense mutation in the PLP gene that is found in all relatives with an unusual form of Pelizaeus-Merzbacher disease. The onset of the disease, age 2.5 years in males and 8–12 years in females, is much later than usual. The clinical course shows deterioration, which is more suggestive of a demyelination rather than the dysmyelination usually associated with PMD. Some degree of dementia or emotional disturbance was noted in several individuals with signs of the disease. Nystagmus was not seen at the time of examination and questioning failed to elicit history of abnormal eye movements during the early years. There are at least four symptomatic females in the family. Examination of the pedigree suggested that inheritance of the disease in this family could be X-linked or autosomal dominant. DNA analysis was undertaken with the expectation that a mutation in PLP would not be found.

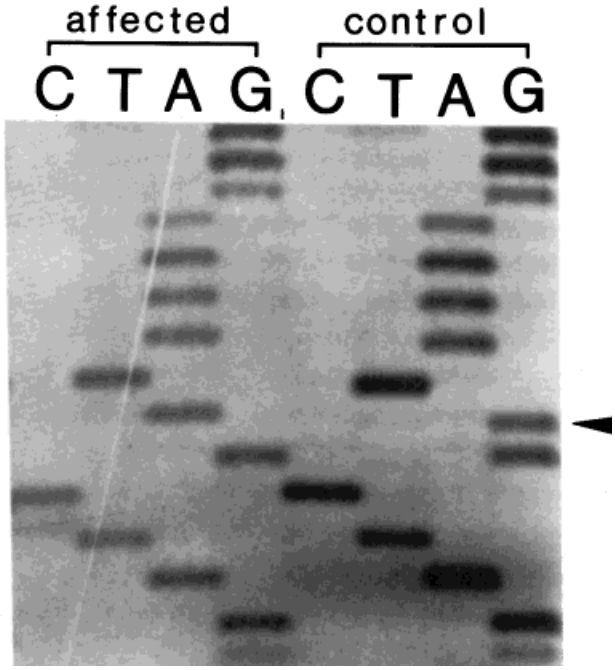

Fig. 3. Partial sequence of exon 3 of the *PLP* gene of an affected male (III-1, Fig. 1) and an unrelated normal control. The G→T transition is indicated by the arrowhead.

The results of the DNA analysis were also unusual. The G→A transition at nucleotide 431 results in a stop instead of tryptophan at codon 144, which is in exon 3B. This is the only known nonsense mutation in PMD not created by a frame shift. This mutation was noted but not described in a review article [Hodes et al., 1993] and, after submission of this manuscript, the same mutation was described in a Japanese family [Osaka et al., 1995]. The highly conserved PLP is cut in half by the stop codon. It is very likely that the mutation is the cause of disease in this family, although the maximum lod score (1.8 at $\theta = 0$) fails to reach significance because of the small number of individuals available for DNA evaluation. However, the absence of this mutation from normal chromosomes and the fact that it is found in all individuals with the clinical picture described strongly suggest cause and effect. Several of the findings in this family deserve additional comment.

Severity of Disease and Possible Relation to DM20

DM20 has the same sequence as PLP save for a string of 35 amino acids that correspond to the second half of exon 3 (called exon 3B) and are absent from DM20 [Schneider et al., 1992]. Work with the mouse allelic mutants *rumpshaker* (*rsh*), jp^{msd} and *jimpy* (*jp*), which show increasingly severe clinical signs of disease, suggests that severity is related to both the abnormality in PLP and to the development and survival

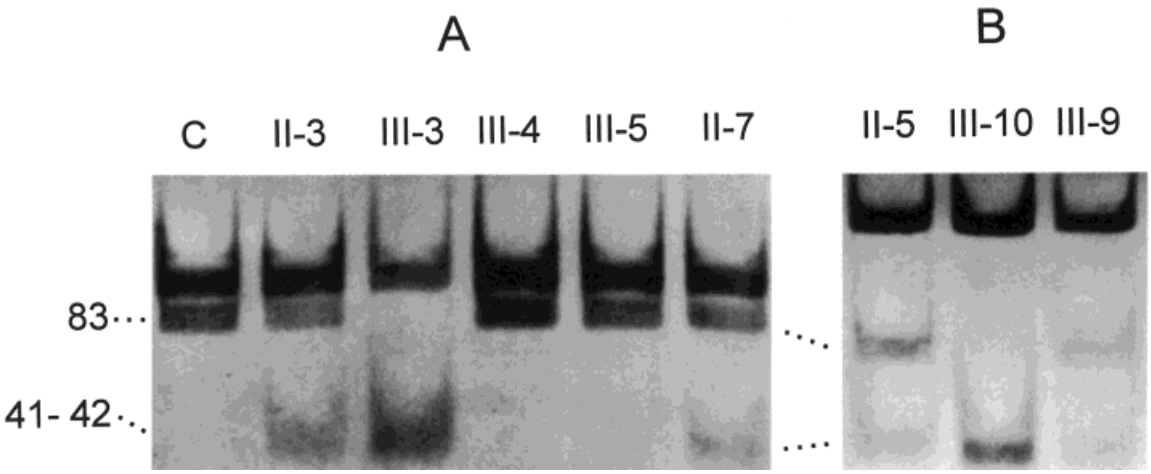

Fig. 4. *Alu*I digestion of amplified DNA from family members. **A**: A ca. 196 bp amplification product (forward primer, 5′GCAGTCAGGCAGATCTTGGCGACT; reverse primer, 5′TCGATTCAAAATCCTGAGGATGATC) digested with *Alu*I results in two fragments; the 83 bp fragment is diagnostic of a normal allele. Presence of the mutation results in cleavage of the 83 bp fragment into pieces of 41 and 42 bp, which are not distinguishable in the gel (e.g., see III-3). C, control male. **B**: A ca. 316 bp amplification fragment (forward primer, 5′TCAAGCTTGTCTACCTGTTAATGC; reverse primer as in A). Again, an 83 bp band represents a normal allele and the 41/42 bp fragments represent alleles containing the mutation.

of oligodendrocytes, the cells that deposit myelin. Oligodendrocyte development and survival is, in turn, dependent on the presence of DM20, which appears before PLP [Ikenaka et al., 1992]. In *rsh*, the mutant amino acid in exon 4 is found in both PLP and DM20, but the latter is presumed functional, whereas the former is not. This mutation (Ile 186Thr) has been found in a family with SPG, the apparently milder form of clinical expression of mutations in *PLP* and which, in this regard, resembles the murine *rumpshaker*. Saugier-Veber et al. [1994] studied a family with SPG that apparently has normal DM20 but abnormal PLP as a result of a mutation in exon 3B. Again, the mildness of disease is attributed to the functional (and in this case, normal) DM20. Saugier-Veber et al. [1994] reinforce their argument by noting that (at the time of their publication) no mutations in 3B were noted in families with PMD.

Absence of nystagmus from all patients in this family and the generally mild disease of late onset are more reminiscent of X-linked spastic paraplegia (SPG) [Johnston and McKusick, 1962] than PMD, whereas other findings as on MRI scans, suggest the latter condition. The mutation is in exon 3B. This should result in a situation like that reported by Saugier-Veber et al. [1994], i.e., no PLP and normal DM20. While the work cited above suggests that clinical severity is related to functional DM20, there is at least one finding that complicates this scenario: there are several families with severe PMD and mutations in exon 3B [Pratt et al., 1991, 1992, 1995; Bridge and Wilkins, 1992] and another family with a mutation at the edge of the region [Nance et al., 1993]. The nonsense mutation we describe, while permitting synthesis of DM20, should result in the absence of functional PLP, unless exon skipping occurs. That would lead to a shorter molecule, which might be partially functional. The effect of these mutations on PLP/DM20 is difficult to test, as the proteins are limited mostly to the central nervous system and models of PLP remain speculative in nature.

Several Affected Females

Although the milder but obvious disease seen in the women in this family could be explained by skewed X inactivation, the large number of expressing females in a kindred with relatively mildly affected males suggests a partly dominant mechanism. The occurrence of affected or symptomatic females in families with Pelizaeus-Merzbacher disease has caused confusion since Merzbacher's report [Merzbacher, 1910]. With the discovery of mutations in PLP as the cause of the disorder in some families, it is now clear that PMD in females need not be due to an autosomal recessive form of the disease [Hodes et al., 1993, 1995]. However, most of the families in these reports have no more than one or two affected females and there can be many unaffected females. In the family reported here, four out of five carrier females (based on DNA results or affected offspring) are symptomatic to some degree. It is possible, but unlikely, that the pattern of X inactivation is skewed severely in all the symptomatic women [Wadelius et al., 1993]. Alternatively, the disorder in this family may be dominant with reduced penetrance. This would suggest that while some disease-causing mutations in PLP are clearly recessive, others may express a dominant phenotype, perhaps via misfolding of the mutant PLP [Schneider et al., 1995]. One might expect that dominant-acting mutations would be restricted primarily to protein-coding regions of PLP.

Other Atypical Manifestations

Personality disorders and dementia, such as are found in this family, have not been considered typical findings in either PMD or SPG. However, the case of

Nance et al. [1993, in press] and a few other cases we are aware of, particularly females, suggest that this is a phenotypic aspect of mutations in *PLP* that warrants further investigation.

Resemblance to Both PMD and SPG

That each PMD family examined has a different mutation in *PLP* is not surprising, as interfamilial variation in clinical findings of PMD has long been recognized. Similarly, SPG is not a single condition, but consists of "pure" (SPG2, #312920) and "complicated" [SPG1, #312900 forms Mckusick, 1992]. Our family falls between SPG and typical PMD, which have been reported to be allelic but distinct conditions [Saugier-Veber et al., 1994; Kobayashi et al., 1994]. It is illustrative that McKusick's original family with "complicated" SPG has the same mutation as the *rumpshaker* mouse [Schneider et al., 1992] and that one baby from this kindred (Naidu et al., unpublished) has the phenotype of PMD. Some phenotypic differences might be the result of peripheral nerve involvement [Kaye et al., 1994], which is not a common finding in PMD. We are convinced that PMD/SPG with mutations in *PLP* is a spectrum of diseases that range from very severe to very mild in clinical manifestation.

Increasingly, we have received diagnostic problems, such as the one presented here, for consideration and have wrestled with the question of which to accept for analysis. As we find mutations in the less typical cases, we have expanded our criteria for acceptance of patients. The discovery of intermediate and atypical cases suggests a simplifying change in the nosology of PMD.

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